MOLECULAR DNA HYBRIDIZATION : AN APPROACH TO DISPUTABLE ISSUES IN FISH TAXONOMY (1)

by

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The method of molecular DNA hybridization suggested as early as 1964 (HOYER, McCARTHY, BOLTON, 1964) has been modified and widely used particularly in the taxonomy of microorganisms. In higher organism systematics, however, it has not yet been extensively applied. To analyze mass systematic data, fairly accurate and relatively simple modifications of this method are being developed in the Laboratory of bioorganic chemistry of the Moscow State University. This is only possible, however, because of the development of techniques for isolating DNA from material fixed in alcool and labelling DNA with tritium *in vitro* by isotope exchange at high (90°) temperature. In our studies, a modified Denhardt method (DENHARDT, 1966) was used, according to which DNA sorbed on nitrocellulose filters is hybridized. Under the incubation conditions adopted, hybridization proceeds mainly in the so-called intermediate frequency fraction or repetitive DNA. It has been shown (MEDNIKOV, 1974) that within Chordata the total DNA hybridization under similar conditions gives a percentage of homology of 5 to 17,5 % for members of different classes, of 17-18 to 45 % (an average of 25-30 %) for members of different orders within the same class, of 50-70 % for members of different families within the same order and over 75 % within the same family.

This type of analysis seems promising for objective determination of taxon rank as can be shown be several of our experiments. The DNA of the cartilaginous fish, *Pterolamiops longimanus*, is hybridized with the DNA of carp, pink

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salmon and sturgeon by 10-15 %, which indicates that Chondrichthyes is a separate class, as distinct from other fishes as fishes are from mammals. Sturgeon and the american paddle-fish, *Polyodon spathula*, were also found to have 10-15 % of their DNA homologous with that of cartilaginous and bony fishes. It follows that the cartilaginous ganoids Chondrostei comprise a separate class, and the validity of grouping Actinopterygii and the whole class of Osteichthyes thus becomes questionable. Interesting results have also been obtained in studying the DNA of the lung fish genus *Protopterus*; the percentage of homologies with the DNA of bony fish was the same as with the DNA of frogs and pigeons and amounted to 12-13 %. This finding is consistent with L.S. Berg's opinion (BERG, 1940) that it is equally valid to classify Dipnoi either with other fishes or with amphibians and birds. We believe that the separation of lower Gnathostoma into different classes is incomplete. Once the DNA of *Latimeria*, Polypteriformes and Holostei has been investigated, the number of classes will increase. It is not inconceivable that Müller's old system (MÜLLER, 1844) will prove to be more valid genetically when its subclasses are raised to the rank of classes. The analysis of the degree to which genetic material diverges among the lower rank taxa leads to fascinating results. It appears, among other things, that the order designated as Perciformes is actually made up of several different orders. Hybridization of the DNA of *Perca fluviatilis* with that of the members of the suborders Anabantoidei, Trichiuroidei, Scombroidei and Notothenioidei reveals that these groups have already diverged to the rank of separate orders. The method of molecular hybridization is efficient in determining the status of infraordinal groups, i.e. families. A taxonomist is often faced with the problem of leaving a divergent species or a group of species in the closest family (as far as characteristics are concerned), or of establishing a new one, even though it may be monotypic.

The usefulness of genotaxonomics can be illustrated by the following example. Coregonid fishes are considered by different scientists either as a separate family or a subfamily of Salmonidae, yet the homology percentage in the DNA of pink salmon and other coregonid species varies from 65 to 58 %. Thus value is of the same order as that of pink salmon and smelt (53 %). Therefore, it is more reasonable to recognize the whitefishes as a separate family Coregonidae, as evidenced by the analysis of the embryological, morphological, biochemical and paleontological characteristics of the suborder Salmonoidei (RESHETNIKOV, 1975).

Discussion of the other results obtained by the molecular hybridization method is beyond the scope of the present report (MEDNIKOV *et al*., 1973). We shall restrict ourselves to the methods of analysing genetic material divergence within related forms, i.e. within the genera of the same family, the species of the same genus and infraspecific forms. In these cases, the general method of molecular hybridization is not applicable since the differences in hybridization percentage are equivalent to experimental error. In view of this, our laboratory has developed a new method according to which the DNA sorbed on a nitrocellulose filter is hybridized not with the total DNA of the reference species but with a fraction of it, which forms thermostable duplexes with T-melting superior to 75°.

On the basis of the shape of the melting curve of hybrid molecules, one may discriminate between the species of one genus and those of related genera. For example, let us consider the analysis of coregonid fishes performed in our laboratory.

The DNA of the anadromous whitefish, *Coregonus lavaretus lavaretus*, was hybridized with the DNA of other coregonids (fig. 1). As seen from the fig. 1, three groups are clearly distinguishable, i.e. three types of DNA hybrid molecular distribution according to thermo-resistance. The first group contains whitefish having inferior mouths (*Coregonus* s. str.) and the second, fishes with upper and terminal mouths (*Leucichthys, Stenodus*), while the thirs monotypic group is represented by the round whitefish (*Prosopium*). A more exact characterization of divergence can be obtained applying the coefficient of divergence (CD). The CD is calculated using the formula : $CD = \Sigma\ d^2 . \log t$, and constitutes the sum of the squares of divergence, multiplied by the logarithm of the resistance temprature of the molecule.

The fig. 2 illustrates the range of gene divergence from the reference species *Coregonus lavaretus* (number I on the fig. 2). In coregonid with inferior mouths (*Coregonus s. str.*), the CD reaches 60 (numbers 2, 3, 4). In cisco (*Leucichthys*) and nelma (*Stenodus*) which are characterized by a different type of hybrid molecular thermostability distribution the CD is 140-160. Round whitefish, *Prosopium*, (8), has the same degree of divergence (157).

For comparison, the CD of more than 200 members of other families (lenok, *Brachymystax lenok* (Salmonidae) and smelt, *Osmerus mordax* (Osmeridae) have been plotted on the graph. Based on these data, the sheme of phylogenetic relations of the family Coregonidae can be suggested. (fig. 3).

In conclusion we should like to cite an example of the analysis of genetic material divergence in intraspecific forms of char. A total of 15 forms of the complicated polymorphic species *Salvelinus alpinus* at different divergence stages has been investigated. The material was collected in North - Eurasia over the territory from the Kola Peninsula to the Far East. The DNA of anadromous char from the Kamchatka river was used as a reference substance. For means of comparison, the CD of *S. leucomaenis* was determined. This species is considered as the only valid one from Eurasia and is distinguished from *S. alpinus* by all taxonomists. The pattern of divergence, presented in fig. 4. indicates that the CD is highest in *S. leucomaenis* (I). In contrast, the CD of all the anadromous chars in the area from the Commander Islands to the Kola Peninsula are practically identical, with the char from the Kola Peninsula being closest to the reference substance, despite the great distance between their area of distribution. This further invalidates the acceptance of *S. malma* as a species allegedly characteristic of the north Pacific. All the other land-locked forms of char from the Taimyr Peninsula, the Kamchatka lakes, the Okhotsk coast and the Baikal region are highly divergent in colour, morphology, and mode of life, and are frequently described as separate species. They should not, however, be so considered, except for the melanistic land-locked form of char from the Kamchatka river (number 7 on the fig. 4) described by K.A. SAVVAITOVA et V.A. MAKSIMOV (1970) and the alpine char from Nachikinskoye Lake in Kamchatka (nº 14) described by K.A. SAVVAITOVA et V.P. VASILIEV (1976). It is noteworthy that these two forms differ significantly from the anadromous char in both morphological characteristics and caryotype structure. They are likely to be recognized as species in *status nascendi*.

The examples cited above illustrate the promising application of the molecular hybridization method in both macro and microsystematics.

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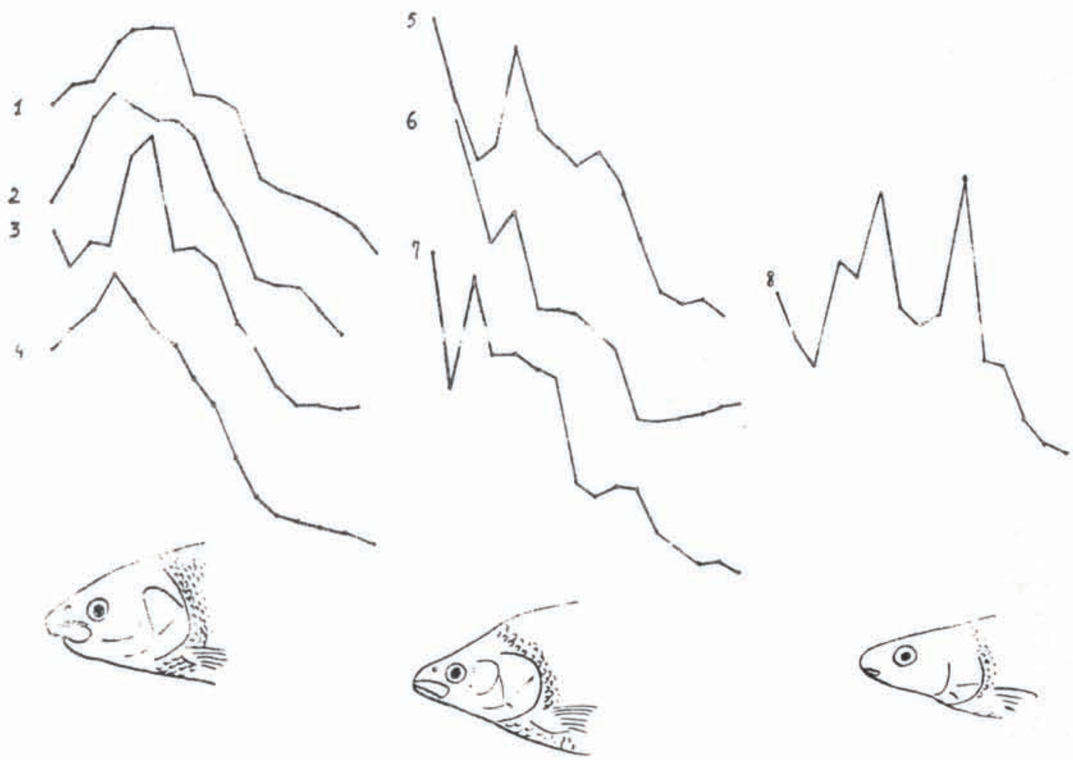

fig. 1 – The three types of DNA hybrid molecular distribution according to thermoresistance.

1 - *C. lavaretus lavaretus*; 2 - *C. lavaretus pidschian*; 3 - *C. nasus*; 4 - *C. muksun*; 5 - *C. omul*; 6 - *C. albula*; 7 - *S. leucichthys*; 8 - *P. cylindraceum*.

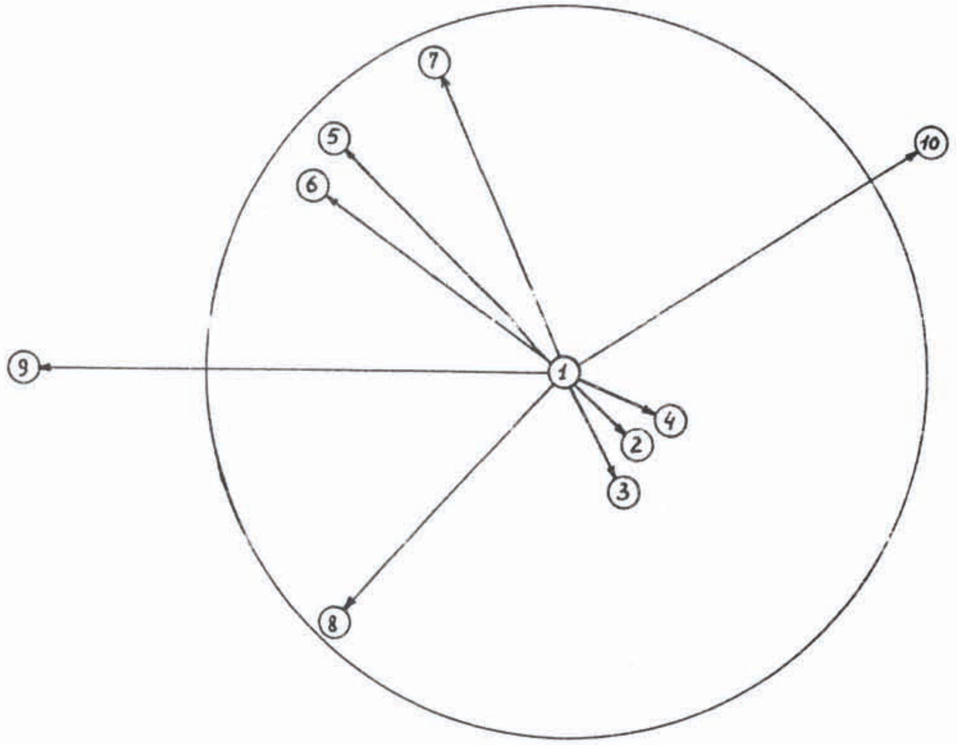

fig. 2 – Relationships among coregonid, salmonid and osmerid fishes according to coefficient of divergence (CD) :
1 - ***C. lavaretus lavaretus***; 2 - ***C. lavaretus pidschian***; 3 - ***C. nasus***; 4 - ***C. muksun***; 5 - ***C. omul***; 6 - ***C. albula***; 7 - ***S. leucichthys***; 8 - ***P. cylindraceum***; 9 - ***B. lenok***; 10 - ***O. mordax***.

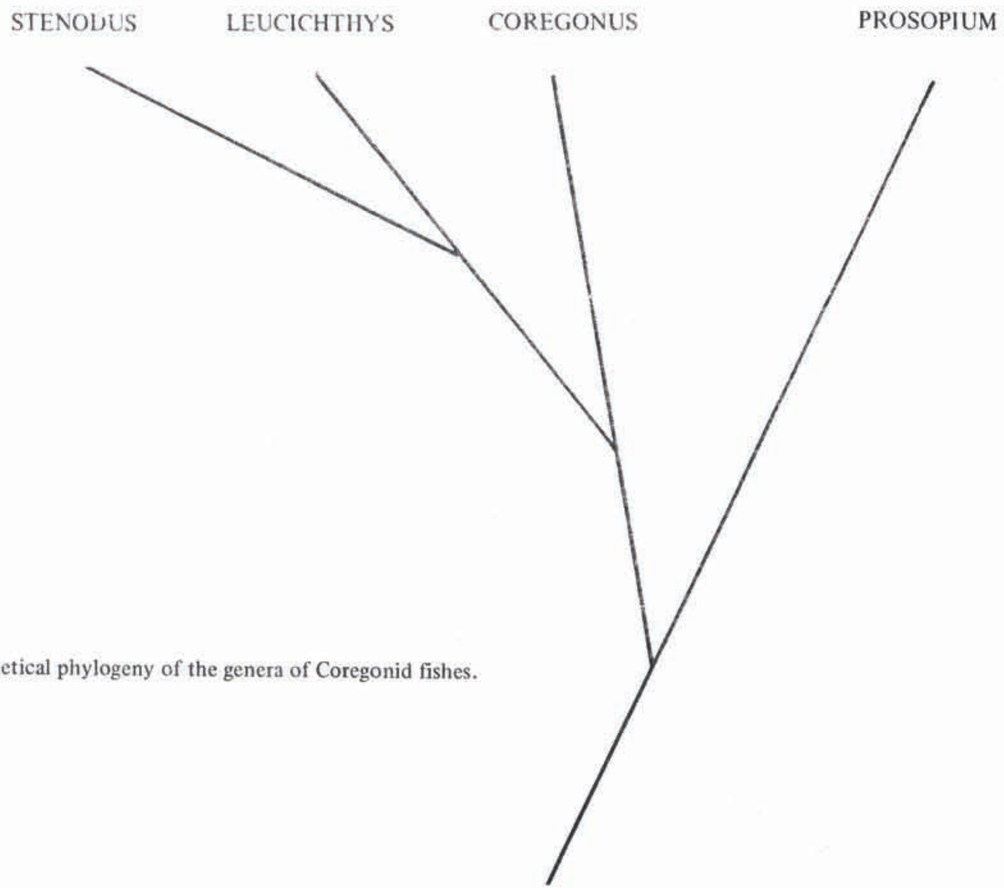

fig. 3 – Hypothetical phylogeny of the genera of Coregonid fishes.

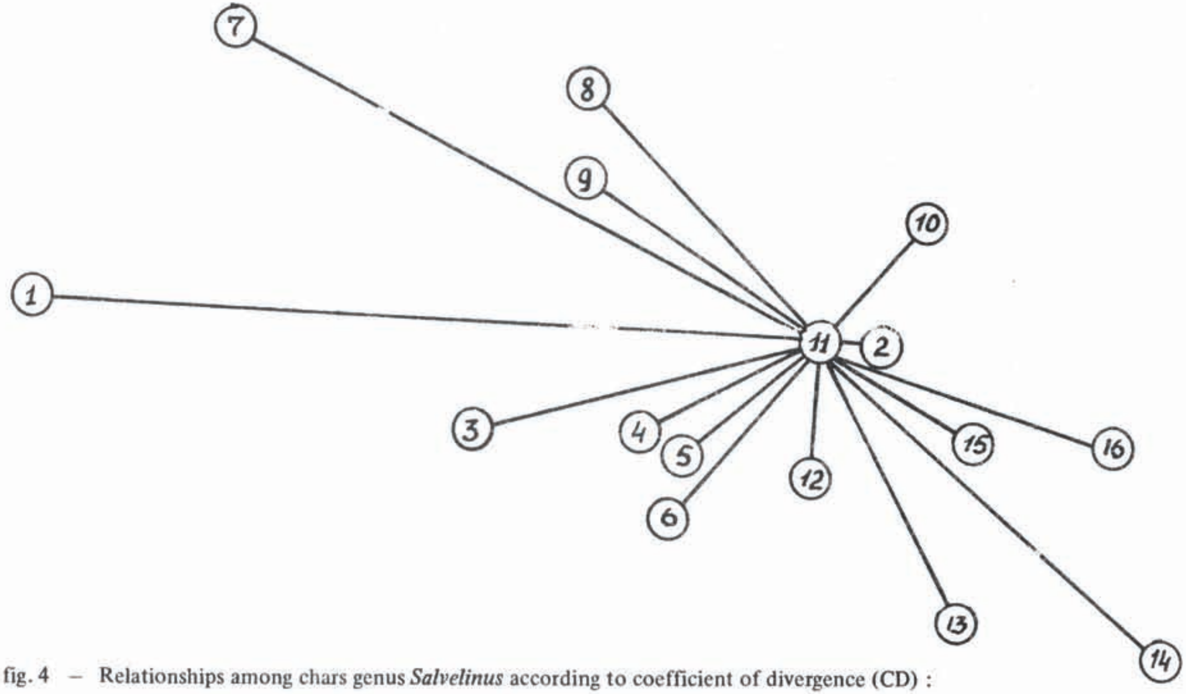

fig. 4 – Relationships among chars genus *Salvelinus* according to coefficient of divergence (CD) :

1 - ***S. leucomaenis***; 2 - ***S. alpinus***, anadromous form (Kolsky Peninsula); 3 - ***S. alpinus***, melanistic lake-river form (Taymir); 4 - ***S. boganidae***, lake-river form (Taymir); 5 - ***S. drjagini***, lake-river form (Taymir); 6 - deepwater lake char (Taymir); 7 - ***S. alpinus***, «Stony» char melanistic river form (Kamchatka); 8 - lake-river predator form (East Kamchatka); 9 - lake-river benthophagous form (East Kamchatka); 10 - anadromous forme (Bering Island); 11 - anadromous form (Kamchatka River); 12 - anadromous form (Bolshaya River, Kamchatka); 13 - lake form (West Kamchatka); 14 - lake-river form (West Kamchatka); 15 - ***S. neiva***, lake form (bassin of Okchota River); 16 - ***S. alpinus erythrinus***, lake-river form (Baikal lake).